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PRINCIPAL INVESTIGATOR: Robert E. Reiter

CONTRACTING ORGANIZATION: University of California, Los Angeles
Los Angeles, California 90095-1406

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6. AUTHOR(S)
Robert E. Reiter

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California, Los Angeles
Los Angeles, California 90095-1406

E-Mail: rreiter@mednet.ucla.edu

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13. ABSTRACT *(Maximum 200 Words)*

Purpose: The overall goal of of our proposal is to develop a new transgenic model of prostate cancer by targeting transformation to prostatic basal cells using the PSCA promoter.
Major Findings: A 9kb region upstream of the PSCA coding region was isolated and demonstrated to drive prostate-specific expression in vitro. This region was subsequently tested in vivo using a GFP reporter construct. Analysis of these mice showed the GFP expression was almost exclusively localized to the prostate. GFP expression in these mice was regulated developmentally, with prominent expression localized to the ductal tips of the prostate. Promoter activity was both responsive to androgen and indepenent of androgen. Recently we have tried to use this promoter to target expression of oncogenes to the prostate. Multiple attempts have failed to produce founders. Analysis of PSCA and GFP expression during embryogenesis showed that PSCA is expressed in multiple peithelial tissues in utero.
Conclusion: The PSCA promoter can target a subset of prostate epithelial cells in vivo, but inducible forms of the promoter may be necessary to deliver oncogenes to these cells.

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Table of Contents

INTRODUCTION

The overall goal of this proposal is to create a new transgenic model of prostate cancer by targeted transformation of basal cells using the Prostate Stem Cell Antigen (PSCA) promoter. The proposal is based on the major hypothesis that prostate cancer arises from malignant transformation of a subset of prostatic basal cells. PSCA is a cell surface antigen whose expression in normal tissues is largely restricted to prostatic basal cells. The specific Aims of our proposal therefore are to 1) clone the PSCA promoter region and identify the sequences capable of driving prostate expression (years 1-2) and 2) to create a model of prostate cancer by targeting SV40 T antigen expression to PSCA-responsive prostatic cells (years 2-3). We have already completed a majority of the work outlined in Aim 1 and have attempted most of the experiments outlined in Aim 2.

PROGRESS REPORT

Specific Aim 1: Delineation of PSCA genomic sequences capable of driving transgene expression in the prostate.

Task 1. Cloning and characterization of mouse and human PSCA genes. The human and mouse PSCA genes were isolated by probing genomic libraries with the respective cDNA clones. 9 and 14kb lambda clones were obtained for the murine and human genes, respectively. These clones were also used to obtain ~120kb BAC clones, in case the lambda clones did not contain all the sequences necessary for prostatic expression. The three known exons of the human and murine PSCA genes were mapped within the lambda and BAC clones and the regions 5' to exon 1 were sequenced. Although other PSCA family members (i.e. Thy-1/Ly-6) contain 4 exons, we have only been able to identify 3 exons in murine and human prostate. The existence of only 3 exons is further supported by the finding of a putative TATA box immediately upstream of the first exon in both the murine and human clones. A potential androgen response element was also identified by sequence analysis of this upstream region. We concluded that the region upstream of exon 1 likely contains the PSCA promoter and enhancer.

Fragments ranging from 1-9kb 5' to exon 1 were isolated by restriction digestion of the lambda and BAC clones. These were cloned upstream of a luciferase reporter gene in order to identify putative promoter/enhancer regions for PSCA. These constructs were transiently transfected into prostatic and nonprostatic cell lines in order to test for activity and prostate specificity. Constructs of 3kb and longer were active in the prostatic cell lines LNCaP and LAPC-4, but not in the kidney cell line 293T. We also saw activity in a bladder cancer cell line, which is consistent with the recent finding in our laboratory that PSCA is expressed at low levels in normal transitional epithelium, as well as in a subset of bladder cancers. Other nonprostatic cell lines tested were negative. These results suggested that the 6 and 9kb human genomic constructs contained the necessary elements for prostate (and bladder) specific expression.

Because we found a potential androgen response element in the PSCA promoter region, we also tested for luciferase activity in prostatic cell lines before and after the

administration of testosterone. As predicted, the PSCA promoter was responsive to androgen, with a 50-100 fold increase in luciferase activity in the presence of testosterone. It is important to note, however, that the PSCA promoter is not dependent on androgen, as it was highly active in prostate epithelial cells (PreC) which do not express androgen receptor. Interestingly, PreC cells express a basal cell phenotype, supporting the hypothesis of this proposal that PSCA is expressed in basal cells, is androgen independent, yet is also androgen responsive.

Task 2. Construction of PSCA-GFP reporter constructs (Please see attached manuscript—Watabe et al.). The 6 and 9kb human PSCA promoter sequences were cloned upstream of the GFP reporter gene using the Clontech pEGFP vector. The activity of this construct was tested in transient transfection assays as described above, with identical results. Stable LNCaP cell lines expressing GFP under the control of the PSCA promoter were also derived for animal experiments.

Tasks 3 and 4. Production and analysis of PSCA-GFP transgenic mice (Please see attached manuscript—Watabe et al.). Based on the hypothesis that the human PSCA promoter would be more tissue-specific than the murine PSCA promoter, the 9 and 6kb human PSCA-GFP constructs were microinjected into C57BL/6XC3H pronuclei to generate PSCA-GFP transgenic mice. Southern blot and PCR assays to detect the presence of the transgene were developed in parallel. Founder mice containing the transgene were bred and analyzed for GFP expression. The F1 generation mice of five DNA-positive founders were analyzed. Mice were analyzed initially by simply dissecting the prostatic lobes and nonprostatic tissues and looking at them directly under the immunofluorescent microscope. Subsequently, individual ducts were microdissected and analyzed, as well as whole mount sections of the individual organs. Two independent lines of mice were found to express GFP in their prostates. Both lines also had detectable GFP in skin. All other tissues analyzed were negative, including stomach, intestine, kidney, seminal vesicle, spleen, liver, salivary glands, brain, lung, thymus, testis and heart. The F1 generation of a single founder line expressed GFP in the bladder; however, the F2 generation lost this expression while maintaining prostatic expression, presumably because of the insertional site of the transgene. As described in the original proposal, the founder line most positive for GFP was maintained and evaluated extensively as follows.

a) Developmental regulation of GFP. GFP expression was studied pre and postpubertally. In 5 day old mice, GFP expression was located in the proximal ducts of the expanding prostate, but not in the distal tips. At 3-6 weeks of age (around the age of puberty), GFP expression was concentrated primarily in the distal tips of the ducts, but extended into the proximal ducts as well. After puberty, at age 14 weeks, all GFP activity was focused in the distal tips, with virtually no expression seen proximally. The significance of these findings is currently being studied, but we hypothesize that GFP is marking a subset of cells which is intermediate in differentiation. Supporting this possibility, we sorted GFP positive and negative cells and did a limited expression analysis. GFP positive cells co-express markers of basal and secretory cells.

b) Hormonal regulation of PSCA-GFP in transgenic mice. Based on our transient transfection data, we predicted that GFP expression would be androgen independent and responsive. Castration experiments of 14 week old PSCA-GFP transgenic mice confirm that GFP expression decreases after androgen ablation. Readministration of androgen leads to reexpression of GFP together with regrowth of the prostatic ducts. As in normal development, GFP expression is broad during active growth, then becomes increasingly restricted to the ductal tips. These result suggest that GFP is not a marker of stem cells, which would be expected to remain following castration, but rather of an intermediate cells which is transiently present during prostate ductal growth.

Specific Aim 2. Use of the SV40 T antigen under the control of the PSCA promoter to create a novel transgenic model of prostate cancer. Our overall hypothesis is that PSCA is a marker of the cell which is transformed during prostate cancer initiation. Aim 1 identified the PSCA promoter elements necessary to direct transgene expression in the prostate. Additional work is necessary to clarify the specific cell type in which GFP is expressed. Based on the preliminary result that GFP is expressed in murine prostate under the control of the current PSCA construct, we made PSCA-T antigen and PSCA-middle T antigen constructs. These were injected into mouse pronuclei, but despite multiple attempts, no positive founders were obtained with either construct. In parallel, we and others looked at PSCA expression during fetal life and found that it is expressed in multiple epithelial tissues during development. These findings suggest that the PSCA promoter would be active during fetal development and that expression of an oncogene during these periods would be lethal. To circumvent these problems, we are currently developing inducible promoters, which can be activated in adulthood. These experiments are beyond the scope of the current grant period.

Key Research Accomplishments

1 Cloning of murine and human PSCA genomic constructs
2 Identification of PSCA promoter/enhancer region
3 In vitro demonstration of promoter activity and tissue specificity
4 Construction of PSCA-GFP transgene
5 Derivation and analysis of PSCA-GFP mice
6 Construction of PSCA-T antigen and PSCA-middle T antigen constructs and injection into mice

Reportable Outcomes

Manuscript submitted on PSCA-GFP mice—Watabe et al.

Conclusions

The overall goal of our proposal was to isolate the PSCA promoter and use it as the basis for creation of a new transgenic model of prostate cancer. To date, we have isolated the promoter, characterized it in vitro and used it to drive expression of a reporter gene in transgenic mice. These results are potentially important because the subset of cells targeted by this promoter may be involved in prostate carcinogenesis. Work to target oncogenes to these cells as proof of this hypothesis has begun, but unexpectedly no founders were obtained. We have shifted strategy to create inducible promoters which can be used to target oncogenes to the prostate.

In Situ Visualization of a Growth Responsive Subset of Epithelial Cells Associated with Prostate Development, Regeneration and Tumorigenesis

Tetsuro Watabe[1], Mark Lin[3], Annemarie A. Donjacour[4], Cory Abate-Shen[5], Gerald R. Cunha[4], Robert E. Reiter[3], and Owen N. Witte[1,2]

[1]Howard Hughes Medical Institute

University of California, Los Angeles

Los Angeles, CA 90095-1662

[2]Department of Microbiology, Immunology & Molecular Genetics

University of California, Los Angeles

Los Angeles, CA 90095

[3]Department of Urology

University of California, Los Angeles

Los Angeles, CA 90095

[4]Department of Anatomy

University of California, San Francisco

San Francisco, CA 94143

[5]Department Center for Advanced Biotechnology and Medicine

University of Medicine and Dentistry of New Jersey

Piscataway, NJ 08854

Corresponding Author:

Owen N. Witte, M.D.

HHMI/UCLA

675 Charles E. Young Dr. S.

Room 5-748 MRL Bldg.

Los Angeles, CA 90095

(310) 206-6411

FAX (310) 206-8822

owenw@microbio.ucla.edu

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Abstract

Benign prostatic hyperplasia (BPH) and prostate cancer are among the most common diseases in aging men and may result from abnormalities in the regulation of stem or progenitor cell growth. An understanding of prostate epithelial development has been hindered by a lack of lineage-specific markers and an inability to identify, isolate and study stem and progenitor cells in vivo. In the present study, the promoter of the prostate stem cell antigen (PSCA) gene was isolated and used to generate transgenic mice in which green fluorescent protein (GFP) is expressed in a subset of prostatic epithelial cells. GFP expression was restricted to the distal regions of the prostatic ducts in adult transgenic mice, a region believed to contain a majority of the proliferative activity in the mature rodent prostate. GFP expression was more widely distributed during the active growth phase associated with puberty and testosterone-induced regeneration of the prostate. Increased levels of GFP expression were also seen in prostate tumors generated by the intercross of PSCA-GFP mice with a transgenic model of prostate cancer (TRAMP). These results suggest that the PSCA promoter is active in a subpopulation of prostatic epithelial cells associated with periods of active cell proliferation, differentiation and transformation. This transgenic mouse line is the first model to enable visualization and isolation of growth responsive cells within the prostatic epithelium and should serve as a useful tool to study normal and neoplastic prostate growth. (232 words)

[*Key words*: prostate; transgenic mouse; prostate stem cell antigen (PSCA); green fluorescence protein (GFP); epithelium; progenitor cell; proliferation; regeneration; tumorigenesis; TRAMP mouse]

INTRODUCTION

Stem cells are defined by their capacity for extensive self-renewal and multilineage differentiation, and are now believed to exist in virtually all tissues (Fuchs and Segre 2000). The colonic crypt, for example, contains stem cells that can give rise to all differentiated lineages within the colon (Potten and Loeffler 1990). These stem cells are located at the base of the crypt and give rise to increasingly committed progenitor cells organized hierarchically from the crypt base up to the mucosal surface. Mammary epithelium, which undergoes dramatic phases of involution and regeneration associated with lactation, has also been shown to contain stem cells (Lochter 1998). Kordon *et al.* demonstrated that a single breast stem cell could give rise to an entire functional mammary gland (Kordon and Smith 1998). Although the exact phenotype of breast stem cells is not known, regeneration experiments suggest that they are scattered throughout the mammary duct and concentrated at the terminal end buds of the mature mammary gland (Lochter 1998).

Epithelial cancers are hypothesized to arise from transformation of stem and progenitor cells. Recent evidence indicates that colon cancers originate from mutated stem or progenitor cells within colonic crypts (Bjerknes 1996). These mutations result in aberrant crypt foci, precursor lesions for invasive colon cancer. Similarly, most breast cancers arise in the terminal ductal structures believed to contain breast stem cells (Wellings et al. 1975; Ishige et al. 1991). These observations highlight the importance of isolating and characterizing stem and progenitor cells in epithelial tissues.

Prostate cancer is the most commonly diagnosed malignancy and a major cause of death in the aging man (Landis et al. 1999). The prostate, like the breast, is a secretory organ that undergoes dramatic changes in response to hormonal fluxes. The prostate requires androgens for ductal growth and maintenance. Castration leads to prostatic involution, with loss of up to ninety percent of epithelial cells due to apoptosis (Kyprianou and Isaacs 1988). Administration of androgen leads to complete regeneration of the prostatic ductal structures (English et al. 1987; Evans and Chandler 1987). These observations are consistent with a stem cell model in which an androgen independent but responsive prostatic stem cell is able to give rise to all differentiated lineages within the mature prostatic epithelium (Bonkhoff and Remberger 1996). The existence of prostatic stem cells is also supported by tissue recombination experiments in which epithelium from the adult rat prostate is grafted under the renal capsule together with urogenital sinus mesenchyme, giving rise to new mature prostatic ducts (Kinbara et al. 1996).

Prostate cancer may originate from transfromation of a stem or progenitor cell. Prostate cancers typically contain malignant populations with both neuroendocrine and secretory phenotypes, the two major differentiated phenotypes of prostatic epithelium, suggesting that they may arise from a common progenitor cell (Bonkhoff and Remberger 1996). Prostate cancers express multiple genes normally restricted to stem or progenitor populations, including bcl-2 (McDonnell et al. 1989; Colombel et al. 1993; Stattin et al. 1996) and telomerase (Sommerfeld et al. 1996). Verhagen and colleagues have identified prostate cancer cells that co-express basal and luminal cell cytokeratins, markers of the putative prostate stem/progenitor and differentiated cells, respectively (Verhagen et al. 1992). The identification of cells that co-express basal and secretory cytokeratins led these investigators to hypothesize that prostate cancer arises from a

prostate lineage intermediate in differentiation between a stem and secretory cell (Bonkhoff and Remberger 1996).

Although multiple lines of evidence point to the existence of prostatic stem and progenitor cells and their potential role in prostate neoplasia, proof of these concepts has been hindered by an inability to visualize or isolate these cells *in vivo*. A single prostate stem cell that is capable of giving rise to a prostatic ductal structure has not been defined. Nor have the progeny of prostatic stem cells, the amplifying or intermediate progenitor cells, been clearly demonstrated to exist *in vivo* in the adult rodent or human prostate. One obstacle is that there are few cell surface markers that can be used to discriminate and isolate individual cell lineages from the prostate.

Prostate stem cell antigen is a GPI-anchored cell surface protein with homology to the Thy-1/Sca-2 gene family (Reiter et al. 1998). Both Thy-1 and Sca-2 are cell surface markers utilized in the study of hematopoietic differentiation (Classon and Coverdale 1994). Sca-2, for example, marks a population of immature thymic lymphocytes intermediate in differentiation between the true stem cell and mature T lymphocytes (Antica et al. 1997). Multiple tissue northern analysis showed that PSCA is expressed predominantly in prostate. PSCA transcripts in normal prostate are restricted to a subset of cells within the basal epithelium, the putative stem cell compartment of the prostate. In addition, PSCA protein is expressed in 80-90% of primary prostate cancers and is overexpressed in prostate cancers that have metastasized to bone (Reiter et al. 1998; Gu et al. 2000). These results suggest that PSCA may be a tissue-specific cell surface marker of a stem or progenitor cell lineage associated with prostate transformation.

Reasoning that the PSCA promoter might provide a means to define a subpopulation of prostatic cells with stem or progenitor cell characteristics in vivo, a transgenic mouse was generated in which the human PSCA promoter was used to control expression of green fluorescent protein (GFP) (Chalfie et al. 1994) expression in the prostate. Consistent with our hypothesis, GFP expression in these mice was restricted to a subset of epithelial cells within the distal tips of the mature prostatic duct. GFP expression increased and broadened during periods of active ductal growth, including puberty and after administration of testosterone to castrate mice. In contrast, GFP expression was barely detectable after castration-induced regression. Finally, GFP was widely and strongly expressed in prostate cancers. These results demonstrate the existence of a growth responsive prostate epithelial cell lineage that is associated with prostate tumorigenesis.

RESULTS

Cloning and characterization of the human PSCA promoter region

A 14kb genomic fragment containing 9kb of sequence upstream from the human PSCA transcription start site was isolated (Reiter et al. 1998) and characterized for promoter activity. In order to determine the activity and tissue specificity of the 5' upstream region, the 9kb fragment was subcloned into a luciferase reporter gene construct and tested for reporter gene expression in a variety of cell lines. Consistent with the known expression of human PSCA in prostate and to a lesser extent in bladder (Reiter et al. 1998; Gu et al. 2000), the 9kb region could drive luciferase expression in prostate (PrEC, LNCaP and LAPC 4) and bladder (HT1376) cell lines, but not in kidney (293T), breast (MCF 7), fibroblast (NIH 3T3) or uterine (HeLa) cell lines (data not shown). These results suggest that the 9kb region contains regulatory elements that are active predominantly in cell lines derived from tissues that can express endogenous PSCA.

In order to characterize the *cis*-elements involved in prostatic PSCA expression further, deletion constructs of the 9kb region were constructed and tested. Two prostatic cell lines, PrEC and LNCaP, were used for these experiments. PrEC cells are primary cultures of normal prostate epithelial cells that do not express androgen receptor (AR) and do not require androgen for growth. They have a predominantly basal cell phenotype—they co-express basal and secretory cell cytokeratins (i.e. CK 5 and 18) and do not express prostate specific antigen (PSA), a marker of terminally differentiated cells. LNCaP is an androgen responsive prostate cancer cell line which expresses both AR and PSA. 1, 3, and 9kb fragments (see Figure 1A) were transiently transfected into these cell lines and assayed for luciferase activity. As shown in Figures 1B and 1C, the 9 kb fragment drove luciferase expression in both PrEC and LNCaP, whereas the 3kb

fragment was active only in LNCaP. These results suggest that sequences between minus 3 and minus 9kb contain *cis*-elements required for PSCA expression in basal-like cells. These results also demonstrate that the 9kb construct is not dependent on androgen for its activity, a unique characteristic which differentiates PSCA from other prostate promoters such as PSA (Henttu et al. 1992; Riegman et al. 2000) and probasin (Rennie et al. 1993; Greenberg et al. 1994) and which is consistent with its expression in basal cells.

Prostate stem and progenitor cells are hypothesized to be both androgen-independent and androgen-responsive, since testosterone leads to prostate ductal regeneration after castration. In order to test the androgen responsiveness of the PSCA promoter/enhancer region, activity of the various constructs was also tested in the presence and absence of exogenous androgen. Both the minus 3 and minus 9kb constructs showed responsiveness to androgen in LNCaP, whereas the minus 1kb construct showed no responsiveness, suggesting that there may be androgen responsive *cis*-elements between minus 1 and minus 3 kb of the PSCA regulatory region.

The 9kb PSCA promoter/enhancer region directs reporter gene expression tissue-specifically in transgenic mice

Transgenic mice carrying enhanced green fluorescent protein (GFP) under the control of the PSCA 9kb promoter/enhancer fragment were generated in order to test the hypothesis that the PSCA promoter could target reporter gene expression to a murine prostate epithelial population analogous to the subset of basal cells in which human PSCA is expressed. We further hypothesized that this population would have characteristics of a stem or progenitor cell. The 9kb fragment was chosen because of its ability to activate gene expression in both AR negative PrEC

cells and AR positive prostate cancer cells. Green fluorescent protein (GFP) was selected because of its ease of detection by immunohistochemistry, fluorescent microscopy and flow cytometry.

Transgenic constructs carrying the 9kb PSCA fragment linked to enhanced GFP (EGFP) (Heim et al. 2000) were injected into pronuclei of C57BL/6 × C3H hybrid mice. Seven pups surviving to maturity were found to contain the transgene by Southern blot and PCR analysis of tail DNA and were bred to C57/Bl6 mice to generate offspring. Eight week old male transgenic and non-transgenic littermates were analyzed for GFP expression by fluorescent microscopy. Prostatic GFP expression was detected in three independent lines, although expression in two of these was weak. The founder with the highest level of GFP expression was maintained and used for further analyses. No extra prostatic GFP expression was seen in the four negative founder lines.

Among the three founder lines with prostatic GFP expression, all had detectable GFP expression in the keratinized skin, but not in the underlying cell layers. One founder line also had GFP expression in the transitional epithelium of the bladder. GFP expression in skin and bladder was detected both in male and female transgenic mice. No other tissue had detectable GFP expression, including lung, stomach, brain, kidney, liver, spleen, bone marrow, seminal vesicle, penis, testis, pancreas or intestine. These results demonstrate that the 9kb PSCA regulatory region can drive reporter gene expression to a very restricted number of tissues *in vivo*.

GFP expression is restricted to the ductal tips of adult mouse prostates

One prediction is that GFP expression in the mouse would mirror PSCA expression in the human prostate and be localized to a subset of prostatic basal cells. However, multiple lines of

evidence suggest that murine and human basal cells may not be equivalent. First, the mouse prostate contains a smaller proportion of basal vs. luminal cells than the human prostate (De Marzo et al. 1998). Second, while basal cells comprise the majority of proliferating cells in the human prostate, most proliferative activity in the mouse prostate is localized to columnar cells lining the distal regions of the prostatic ducts (Lee et al. 1990; Sugimura et al. 1996). Third, secretory activity in the murine prostate is maximum in intermediate regions of the duct, compared to humans in which no such regional heterogeneity has been reported (Lee et al. 1990). Finally, the proximal ducts of the mouse prostate express Cathepsin D, a marker of apoptosis (Lee et al. 1990). No equivalent zone has been demonstrated in the human prostate. These observations have led Lee and others to propose a model in which the mouse prostate is organized along a distal to proximal axis, with the distal regions containing stem and/or progenitor cells that migrate proximally to give rise first to terminally differentiated secretory cells, then to cuboidal cells destined for apoptosis (Fig. 2A).

In order to localize the site of GFP expression in transgenic mice, lateral prostates from 8 week old (postpubertal) transgenic and non-transgenic mice were harvested and examined under the fluorescent microscope. As is shown in Figure 2B, GFP expression was restricted to the distal region of each individual ductal structure in transgenic mice. No GFP expression was seen in the intermediate or proximal regions of the mature duct, or in non-transgenic littermates. These results demonstrate that the human PSCA promoter directs transgene expression to a subpopulation of murine prostate epithelial cells, which are located in a region of the prostate proposed to contain a high proportion of stem or progenitor cells.

GFP expression is detected early in prostate ductal morphogenesis

PSCA promoter directed GFP expression in the distal ductal tips may mark a progenitor or stem cell lineage of prostatic epithelium. Because the pool of stem or progenitor cells is expected to vary during prostate development, we next evaluated whether the pattern of GFP expression changes during development. We examined fetal tissue to see if GFP expression could be detected at day 16-18 dpc, when the prostate arises as a bud off of the urogenital sinus [diagrammed in Fig. 3A and B; Cunha, 1994 #9370]. Although PSCA promoter directed GFP expression could not be detected by fluorescent microscopy at this timepoint, it could be detected immunohistochemically using an anti-GFP antibody at day 16 dpc in the epithelium of the urogenital sinus (Fig. 3C). No GFP expression was detected in the solid mass of outgrowing prostatic buds first visualized at day 18 (Fig. 3D). However, GFP expression could be detected in the proximal canalizing region of the prostatic buds (Fig. 3D), suggesting that GFP expression may identify a subset of prostatic cells that are differentiating during embryogenesis.

In order to characterize GFP expression in the urogenital sinus at 18 dpc further, we compared expression of NKX 3.1 with GFP. NKX3.1 is a prostate specific homeobox gene and is the earliest known marker of prostatic epithelium (Bhatia-Gaur et al. 1999). NKX3.1 expression was detected in the outgrowing prostatic buds and in the peripheral region of the urogenital sinus epithelium, in a manner which was complementary to the pattern of GFP expression (Fig. 3E). NKX3.1 appears to mark a rapidly proliferating population of cells that initiate prostate ductal formation, whereas GFP correlates with the early stages of ductal canalization.

GFP expression is developmentally regulated

In rodents, the prostate undergoes extensive ductal outgrowth and branching during the first three weeks postnatally (Cunha 1994). At one week of age, PSCA promoter directed GFP expression was detected at low levels by fluorescent microscopy and localized to all but the distal-most regions of the prostatic duct, where canalization had not yet occurred (Fig. 4A). During puberty (i.e. 5 weeks of age), GFP expression increased dramatically and was detectable in all but the most proximal regions of the duct (Fig. 4B). In contrast with 1 week old mice, GFP expression at 5 weeks was now present in the distal tips of the ducts. At 8 weeks, following puberty, GFP expression became restricted to the distal regions of the prostatic ducts (Fig. 4C) and this pattern became even more accentuated in the mature 14 week old gland (Fig. 4D). These results demonstrate that GFP marks a subpopulation of prostatic cells which are tightly regulated during prostatic development. In addition, these results suggest a model of prostate epithelial development characterized by the presence of at least three distinct cell subsets. NKX3.1 marks the earliest subset of prostatic epithelial cells as they emerge off the urogenital sinus and before ductal canalization occurs. GFP identifies a population of cells associated with prostatic growth and development. These cells appear to be committed towards differentiation, as they are found exclusively in columnar cells lining new glands. The third cell subset is a GFP negative population of cells found in mature ducts. These cells, localized to intermediate regions of the ducts, may be terminally differentiated and may contribute to the bulk of prostatic secretions.

Regulation of GFP expression during prostatic involution and regeneration

Tissue regeneration in the liver (Michalopoulos and DeFrances 1997; Petersen et al. 1999) and prostate (English et al. 1987; Evans and Chandler 1987) requires rapid cell proliferation and differentiation of multipotent stem cells. In order to study GFP expression during controlled

periods of tissue regeneration, 14 week old transgenic mice were examined after castration and following re-administration of exogenous androgen. Withdrawal of androgen by castration led to prostatic involution, as evidenced by a 90% loss in mean prostatic weight (Kyprianou and Isaacs 1988). Whereas GFP expression was detected in the distal regions of prostatic ducts in 14 week old transgenic mice (Fig. 5A), there was loss of detectable GFP fluorescent activity two weeks after castration (Fig. 5B). Because these residual epithelial cells are known to replenish the prostate after androgen administration, these results suggest that PSCA promoter-driven GFP expression is low or absent in the mitotically quiescent stem cell population.

Prostate ductal regeneration was induced by administration of testosterone pellets. As predicted, mean prostatic weight returned to normal by 4-5 weeks, indicative of ductal regrowth. GFP expression was detected in the distal regions of the newly regenerating ducts beginning one day after androgen treatment (Fig. 5C) and both strengthened and broadened at one week (Fig. 5D). Over the ensuing four weeks, GFP expression became increasingly restricted to the distal regions of the maturing ducts (Fig. 5E), similar to the pattern seen during normal development (see Fig. 4). GFP expression was not induced simply by androgen, as there was no increase in GFP expression when exogenous testosterone pellets were administered to intact mice for one week (Fig. 5F). These results support the earlier observation that GFP expression is tightly regulated during the processes of ductal growth and differentiation. Likewise, these results are consistent with the hypothesis that GFP expression in transgenic mice marks an epithelial lineage associated with active proliferation and differentiation.

Because GFP expression was restricted to a subset of cells associated with proliferation and differentiation, we predicted that these cells would express genes associated with these two processes. Lateral prostates from 8 week old non-transgenic or transgenic mice were excised, dissociated and subjected to flow cytometry. As shown in Fig. 6A and B, a GFP positive population of cells could be detected exclusively in transgenic mice. GFP positive and negative cells were sorted from the transgenic mice, processed for RNA, and then analyzed for gene expression by RT-PCR. ß-actin was used for normalization. GFP expression was detected, as expected, only in the GFP positive population, confirming the efficiency of sorting. Likewise, murine PSCA expression was detected differentially in the GFP-positive population, suggesting that the human PSCA promoter drives GFP expression exclusively to cells that express endogenous murine PSCA transcripts. Estrogen receptor β (ERβ) is differentially expressed in the distal regions of rodent prostatic ductal structures (Prins et al. 1998). Since GFP expression is restricted to the distal regions of prostatic ducts in the transgenic mice, the differential expression of ERβ in GFP positive cells confirmed that this sorting enriched the subpopulation of cells that are localized in distal regions of prostatic ducts. CD44 is a cell surface antigen whose expression in human prostate is restricted to the basal layer (Liu et al. 1997b). Differential expression of CD44 in GFP positive cells suggest that GFP expression define the cell populations that correspond to subsets of basal cells within human prostatic epithelium. Probasin, a marker of differentiating or terminally differentiated secretory cells of the mouse prostate (Johnson et al. 2000), was detected in both GFP-positive and negative cells. This result is consistent with the observation that GFP expression is associated with early stages of differentiation. We also evaluated the expression of ETS2 (Liu et al. 1997a), bone morphogenetic protein receptor type 1A and 1B (BMPR1A and 1B) (Ide et al. 1997) in GFP-positive and

negative cells. ETS2 is a transcription factor whose expression in normal human prostate is restricted to basal cells. ETS-2 expression is increased in some cancers and has been associated with maintenance of the transformed phenotype (Sementchenko et al. 1998). BMPR1A is a positive regulator of prostate cancer cell growth, while BMPR1B is a negative regulator. As shown in Figure 6C, ETS2 and BMPR1A were differentially expressed in GFP positive cells, while BMPR1B was not. The differential expression of these two genes in GFP positive cells suggests that GFP expression correlates with the proliferative activity of prostatic cells. The finding that GFP-positive cells co-express markers of secretory (i.e. probasin) and basal cells (i.e. ETS2, CD44) also suggests that PSCA promoter-driven GFP expression marks a subpopulation of cells which is intermediate in differentiation between a stem and terminally differentiated cell.

GFP expression is elevated during prostate tumorigenesis

The cellular origin of prostate cancer is not known. In order to test the possibility that cancer may arises from the cell lineage marked by GFP expression in PSCA-GFP mice, these mice were mated to a transgenic mouse model of prostate cancer (TRAMP) (Greenberg et al. 1995). TRAMP mice contain the SV40-T antigen under the control of the androgen dependent rat probasin promoter. In these mice, T antigen becomes detectable by 8 weeks of age in the secretory epithelium of the dorsolateral and ventral prostates. Mice develop prostatic intraepithelial neoplasia (PIN—a precursor of prostate cancer) by 10 weeks, then proceed to develop invasive tumors. By 28 weeks, 100% of TRAMP mice develop metastatic disease to lymph nodes, lung and other soft tissues (Gingrich et al. 1996). Importantly, TRAMP tumors have been shown to contain many of the same molecular abnormalities commonly seen in human

tumors, such as loss of E-cadherin (Gingrich et al. 1996) and elevated levels of IGF (Kaplan et al. 1999), indicating that these tumors may be good models for studying prostate cancer.

Female TRAMP mice were bred to PSCA-GFP males. Three litters containing male GFP-/TRAMP-, GFP+/TRAMP- and GFP+/TRAMP+ transgenic mice were sacrificed at 20-25 weeks of age and examined for GFP expression. As shown in Figure 7A, the prostatic lobes and seminal vesicles were dramatically enlarged and irregular in TRAMP+/GFP+ mice, suggestive of tumorigenesis. At this age, little if any GFP expression was detectable in the lateral or dorsal prostate from GFP+/TRAMP- mice (Fig. 7B and C). In contrast, moderate to strong GFP expression was seen throughout the ducts of dorsal and lateral prostates of TRAMP+/GFP+ mice (Fig. 7B and C).

In order to characterize these tumors further, prostates from GFP-/TRAMP-, GFP+/TRAMP- and GFP+/TRAMP+ mice were sectioned and stained with anti-GFP antibody. While GFP expression was not detectable in the sections obtained from GFP+/TRAMP- prostates (Fig. 7D), GFP+/TRAMP+ prostates clearly contained invasive tumors which were strongly positive for GFP expression (Fig. 7E). Prostate cancers obtained from GFP-/TRAMP+ mice did not stain positive for GFP (Fig. 7G), nor did GFP+/TRAMP+ tumors stain positively with a rabbit IgG control (Fig 7F). These results suggest that GFP expression marks a subset of cells correlated with tumorigenesis in this well defined transgenic mouse model of prostate cancer. These results also suggest that the PSCA promoter may be activated in cancer, since GFP expression was markedly increased in cancer when compared to the normal gland.

DISCUSSION

The human PSCA regulatory region

The human PSCA regulatory region is induced by androgens in both LNCaP cells and in PSCA-GFP transgenic mice. GFP expression diminished after castration, then increased upon re-challenge with testosterone. Similarly, PSCA promoter activity increased after exogenous addition of androgen to LNCaP cells. Analysis of deletion constructs suggests that androgen-responsive elements reside between minus 3 kb and minus 1 kb of the PSCA regulatory region. It is not known whether androgen directly regulates PSCA by binding to the PSCA promoter or whether the increased acitivty is related to the positive proliferative effect of androgen on LNCaP or the casrated prostate. No consensus androgen response elements (AREs) are found in the 2 kb PSCA promoter sequence to which the activity maps. Also, there was no increase in GFP expression in transgenic prostates when exogenous androgen was added to intact male mice, suggesting that androgen may not regulate the PSCA promoter directly. During prostate development, it has been shown that some androgenic effects, such as ductal morphogenesis, epithelial growth, and secretory cytodifferentiation, do not require the presence of intraepithelial androgen receptors. Analysis of chimeric prostates constructed with androgen receptor-positive wild-type mesenchyme and androgen receptor-negative Tfm (testicular feminization) bladder epithelium showed that androgen can mediate its effects via a paracrine factors released from the stroma (Cunha et al. 1986). Similar indirect effects may explain the androgen inducibility of the PSCA promoter.

Unlike other prostate-associated genes such as PSA, PSCA expression is not dependent on androgen and is expressed in AR negative PrEC cells (R. Reiter, unpublished data). Consistent

with this observation, the 9kb PSCA regulatory region was active in PrEC cells. These results suggest that the 9 kb PSCA promoter is regulated by mechanisms different than those that regulate the promoter of human PSA (Henttu et al. 1992; Riegman et al. 2000) or rat probasin (Rennie et al. 1993; Greenberg et al. 1994), whose expression is strongly dependent on androgen. These observations suggest that the PSCA promoter may serve as an alternative promoter to be used for gene therapy or replicating viral therapies of prostate cancer, particulalry in those tumors that do not express AR or for which the PSA promoter is inactive (Gotoh et al. 1998) (Hobisch et al. 1996).

GFP expression in PSCA-GFP transgenic mice is a novel marker of prostate development

The isolation and characterization of prostate epithelial lineages has been hindered by a lack of markers of normal development. In the present study, we demonstrate that GFP expression in PSCA-GFP transgenic mice identifies a subpopulation of prostatic epithelial cells that is associated with proliferation and differentiation during normal development and regeneration. We have also shown that this GFP positive cell population can be isolated by FACS and co-expresses markers associated with proliferation and differentiation of the prostatic epithelium. These results support a model in which PSCA promoter-driven GFP expression marks a population of cells of intermediate differentiation. Similar strategies have been used to identify developmental lineages of the lung (Hansbrough et al. 1993), small intestine (Bry et al. 1994), cardiovascular system (Schlaeger et al. 1995) and nervous system (Yamaguchi et al. 2000). Gordon and colleagues generated a transgenic mouse line that expresses a β-galactosidase reporter gene in a subset of differentiating cells during early lung development (Hansbrough et al. 1993). The mouse lung arises as an outgrowth of the embryonic foregut by embryonic day 10.

Reporter gene expression in these mice was detected throughout the pulmonary endoderm on day 11 dpc, then extinguished in a proximal-to-distal wave, which parallels the order of cytodifferentiation of the pulmonary endoderm. In day 16 dpc embryos, reporter gene expression was restricted to the distal region of epithelial tubules and by birth to scattered cells located in alveoli. This expression pattern is intriguing because it is similar to the GFP expression pattern in PSCA-GFP transgenic mice, suggesting that differentiation takes place in a similar manner during formation of epithelial ductal structures of various tissues.

This pattern of differentiation is also observed in mammary epithelium. The mammary gland originates during embryogenesis from a thickening of ectoderm that invaginates into the surrounding mesenchyme to form the mammary anlage consisting of short ductal structures with small end buds. During puberty, when the level of estrogen increases, elongation of ducts takes place as a result of rapid growth in terminal buds, which is similar to that which occurs in prostatic ducts (Sugimura et al. 1996). In the resting mature mammary gland, there are dynamic estrogen-dependent changes in tissue organization accompanied by peaks of proliferation and apoptosis (involution). During mammary duct growth, the posterior region of the terminal buds provides a supply of differentiating cells for ductal elongation. Regeneration experiments have shown that mammary stem cells are scattered throughout the ductal structures and are concentrated in the terminal end buds of the mature mammary gland (Lochter 1998). These observations suggest that prostate and breast are similar not only in their dependence on steroid hormones, but also share a common functional ductal organization.

GFP expression and prostate cancer

GFP expression is increased dramatically in prostate cancers generated by the intercross of TRAMP and PSCA-GFP mice. One possiible explanation for this finding is that the PSCA promoter was activated by SV40T antigen. To test this possibility, LNCaP cells were transfected with the luciferase reporter gene carrying the PSCA 9 kb promoter in the absence or presence of an expression vector encoding SV40T antigen. The expression of SV40T antigen did not activate the PSCA promoter (data not shown), suggesting that the elevated GFP expression was not induced directly by SV40T antigen. It is possible, however, that a signal downstream of SV40 T antigen, which is associated with transformation, could have activated GFP expression.

A second possibility is that the GFP-positive subpopulation of cells was the target of transformation in this model. Transformation and resulting maturation arrest of these cells could explain the expansion in GFP positive cells in these tumors. Supporting this possibility is the finding that GFP-positive cells co-express probasin, suggesting that SV40 T antigen was activated in and could have transformed these cells. This model is consistent with a stem or progenitor cell model of prostate carcinogenesis (Bonkhoff and Remberger 1996).

A third possibility is that GFP expression was reactivated by dedifferentiation of transformed GFP-negative secretory cells. As in other systems, it is difficult to discriminate between dedifferentiation of differentiated cells and expansion of undifferentiated cells. We are currently trying to resolve this dilemma by delivering oncogenes directly to PSCA promoter-driven cells and by studying GFP expression in other transgenic models of prostate cancer (cryptidin-

SV40TAG; Guardian et al. 1998) (Di Cristofano et al. 1998; Podsypanina et al. 1999) (Bhatia-Gaur et al. 1999).

In summary, PSCA-driven GFP expression in transgenic mice has allowed clear visualization of a subpopulation of prostatic epithelial cells associated with growth, regeneration and transformation of the prostate. This model should provide new insights into both normal and neoplastic growth of the prostate.

Material and methods

Construction of expression plasmids

A human PSCA genomic clone has been described previously (Reiter et al. 1998). The 14 kb PSCA genomic DNA fragment was subcloned into pBluescript II SK (Strategene) This construct was used to generate the fragment carrying 9 kb of 5' flanking sequence and 5 bp of untranslated region by high fidelity PCR (Roche) using the *Hind*III/3' primer and T7 primer. The PCR fragment was digested by HindIII to obtain a 2 kb fragment flanked by a blunt end and a HindIII site, and a 7 kb fragment flanked by HindIII sites. The pGL3-PSCA(-9 kb) vector was constructed from those two fragments by inserting them into the *Sma*I and *Hind*III sites of the promoterless luciferase vector pGL3-basic (Promega). The pGL3-PSCA(-3 kb) and pGL3-PSCA(-1 kb) vectors were constructed by restriction digestion of pGL3-PSCA(-9 kb) by *BamH*I and *Kpn*I, respectively, followed by self ligation.

In order to construct the PSCA (9 kb)-GFP transgenic vector, the fragment carrying 9 kb of 5' flanking sequence and 5 bp of untranslated region was obtained by high fidelity PCR (Roche) using the *EcoRI*/3' primer and T7 primer. The pEGFP-PSCA(-9 kb) vector was constructed from the EcoRI digested PCR fragment inserted into the *EcoR*I site of a promoterless EGFP vector, which was constructed by removal of the CMV promoter sequence from the pEGFP-N1 vector (Clontech). The *Hind*III/3' primer and *EcoRI*/3' primer contained *Hind*III and *EcoR*I restriction sites at their 5' termini, respectively. Sequences of the PCR primers are listed below. *Hind*III/3' primer: 5'-GGGAAGCTTGCACAGCCTTCAGGGTC-3'; *EcoRI*/3' primer: 5'-GGGAATTCGCACAGCCTTCAGGGTC-3'.

Cell cultures, DNA transfection and luciferase assay

LNCaP cells were originally obtained from the American Type Culture Collections (Rockville, MD) and primary normal prostate epithelial cells (PrEC) were purchased from Clonetics (Biowhittaker, San Diego, CA). LNCaP cells were maintained in RPMI 1640 supplemented with 5% FCS. For examination of androgen induction in transfection experiments, the synthetic androgen R1881 (DuPont NEN, Boston MA) was added to a final concentration of 10 nM. PrEC cells were cultured in the proprietary growth medium (PREGM) from Clonetics. All cells were maintained in a humidified incubator at 37°C and 5% CO2.

Cells were transfected using FuGene6 (Boeringer Manheim) according to the lipofection method described in the manufacturer's protocol. Briefly, 2×10^5 cells per 25-cm^2 flask were co-transfected with 0.4 μg of the pGL3 firefly luciferase reporter construct carrying the appropriate PSCA promoter sequence and 0.4 μg of the pRL-TK *Renilla* luciferase reporter construct carrying the HSV-TK promoter using 2 μl of FuGene6. Transfections were performed in triplicate and repeated at least three times. Cells were collected approximately 48 hours after transfection with 1× tissue lysis buffer (Dual-Luciferase Reporter Assay System; Promega, Chicago, IL). Firefly and *Renilla* luciferase activities were measured by luminometry. For each assay, 10 μl of cell lysate was used. The firefly luciferase activity was finally adjusted according to the *Renilla* luciferase activity of 10 μl of cell lysate.

Generation of transgenic mice and animal handling

Transgenic mouse lines were produced by injecting the purified *Sac*I and *Afl*II fragment into mouse fertilized eggs derived from intercrosses of (C57BL/6 × C3H) hybrid mice. Founders were identified by Southern blot analysis of tail DNAs using EGFP cDNA as a probe. Founder mice carrying transgenes were bred to C57BL/6 mice to generate offsprings that were subject to GFP analysis. Mouse studies were performed according to the guidelines set forth by the UCLA Animal Research Committee.

The transgenic mouse line was maintained by mating animals that were hemizygous for the transgene to C57BL/6 mice. Prostates were harvested as described (Greenberg et al. 1994). Developmental studies were initiated by mating a male hemizygous transgenic mouse to a female C57BL/6 mice. The day the vaginal plug was first noted was scored as day 0 of gestation. Mice were surgically castrated using standard techniques. For hormone restoration analysis, a testosterone pellet was administered subcutaneously. Additional testosterone pellets were placed every two weeks for appropriate groups of mice.

Histology and immunohistochemistry

Tissues from transgenic and non-transgenic mice were dissected and mounted in a plates supplied with PBS. To visualize GFP, an Olympus microscope was equipped for epifluorescence with a BP490-FY 455 filter set. For immunohistochemistry, freshly taken tissues were fixed in 10% formallin for 12 hours at 4°C, followed by paraffinization. Sections were processed for immunohistochemistry using StreptABComplex/HRP kit (DAKO, Denmark) according to the manufacturer's protocol. Polyclonal antibody against GFP (Molecular Probes)

was used at a concentration of 10 ng/μl. The sections were counterstained in hematoxylin and mounted in Permount. The anti-NKX3.1 antibody was prepared against bacterially expressed protein in rabbits.

Single cell suspension and flow cytometry

Prostate tissues were minced and digested with collagenase I (Sigma) at a concentration of 1 mg/ml in DMEM medium supplemented with 10% FCS for one hour at room temperature. Large debris were excluded by 0.45 μm cell strainer. Single-cell suspensions were analyzed by FACS Vantage (Becton Dickinson) after exclusion of small debris by defining a gate based on forward- and side-scatter profiles. Four-quadrant, two-dimensional plots were generated by logarithmic amplification of fluorescence emitted by single cells. Control (double negative) quadrants were determined by evaluation of the cells from non-transgenic mice. Subpopulations of prostatic cells were purified by sorting with FACS Vantage (Beckton Dickinson).

Gene expression analysis

The technique of reverse transcriptase-polymerase chain reaction (RT-PCR) and cDNA amplification was applied to detect the expression of marker genes in the sorted cell populations. Briefly, polyA+ RNAs were obtained from 1×10^5 sorted cells using QuickPrep Micro mRNA Purification Kit (Amersham Pharmacia Biotech). All the RNAs were coverted to cDNA using the SMART cDNA construction kit (CLONTECH), followed by PCR-based amplification of cDNA. Sequences present in the cDNA were detected by PCR with gene-specific primer pairs and agarose electrophoresis of the reaction products. PCR cycle number for each marker gene

was used in linear range. To normalize the amounts of input cDNA, amplification of β-actin cDNA was used as a control. DNA sequences of the primer pairs are given as follows:

β-actin-5, GACTACCTCATGAAGATCCT

β-actin-3, GCGGATGTCCACGTCACACT

GFP-5, CTGGTCGAGCTGGACGGCGACG

GFP-3, CACGAACTCCAGCAGGACCATG

mPSCA-5, TTCTCCTGCTGGCCACCTAC

mPSCA-3, GCAGCTCATCCCTTCACAAT

Estrogen receptor β-5, GTCCTGCTGTGATGAACTACAGTG

Estrogen receptor β-3, CTCTTGGCGCTTGGACTAGTAAC

CD44-5, ATCACCGACAGCACAGACAGAATCCCT

CD44-3, ATCTGATTCAGATCCATGAGTGGTATG

probasin-5, ATCATCCTTCTGCTCACACTGCATG

probasin-3, ACAGTTGTCCGTGTCCATGATACGC

ETS2-5, TCGGCTCAACACCGTCAATGTCAA

ETS2-3, TTCTGTATCAGGCTGGACGCCCAG

BMP type I receptor A-5, GCATAACTAATGGCCATTGC

BMP type I receptor A -3, CCTGCTTGAGATACTCTTAC

BMP type I receptor B-5, GCAGCACAGATGGGTACTGC

BMP type I receptor B-3, TCTCATGCCGCATCAGGACC

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Figure legends

Figure 1.

Characterization of human PSCA promoter sequence in PrEC and LNCaP cells.

(A) Schematic representation of the structure of human PSCA gene and the luciferase reporter constructs containing the DNA fragments encoding the PSCA gene. PSCA gene has three exons. Protein coding and untranslated regions are represented by solid and open boxes, respectively. An untranslated region (open box) and various 5' flanking sequences (solid line) of the PSCA gene were subcloned in front of luciferase reporter gene (shaded box) as described in material and methods.

(B and C) PrEC (B) and LNCaP (C) cells were transiently transfected with various PSCA-LUC constructs as described in material and methods. Relative luciferase activity was calculated according to the luciferase activity driven by CMV promoter. S.E. of relative activity is represented by a horizontal stripe. (D) LNCaP cells were transfected with various PSCA-LUC constructs in the absence or presence of synthetic androgen, R1881. Activity in the presence or absence of R1881 was represented by an open or solid bar, respectively. Hormone induction is given at the left of the bars.

Figure 2.

GFP expression is restricted to the distal region of prostatic ductal structures in PSCA-GFP transgenic mice.

(A) Simplified diagram of a murine adult prostate. Ductal structures can be divided into three regions along their length by their cellular characteristics as described in text.

(B) GFP expression in the lateral prostate from an 8 week old PSCA-GFP transgenic mouse (top panel) or a non-transgenic littermate (bottom panel). Left and right panel represent transillumination and GFP fluorescence images, respectively, of same prostate tissue. Prostates were placed so that proximal ends to urethra are on the right of the panel. Scale bar, 200 μm.

Figure 3.

GFP expression during the embryonic development of prostate in PSCA-GFP transgenic mice.

(A, B) Schematic diagrams of sagittal sections of male urogenital sini, showing the appearance of prostates prior to the onset of ductal budding (16 dpc, A), and an early stage in the formation of prostatic ductal structures (18 dpc, B). M (blue), urogenital sinus mesenchyme; E (green), urogenital sinus epithelium; P (green), prostatic duct.

(C, D) Sections of the 16 dpc (C) and 18 dpc (D) transgenic urogenital sini immunostained with an anti-GFP antibody (brown) and counterstained with hematoxylin (dark blue). GFP expression was detected in the urogenital sinus epithelium, and was not detected in the outgrowing prostatic ducts. Note that GFP was detected in the proximal canalizing region (D, magnified image). A serial section immunostained with control rabbit IgG showed no signal (data not shown).

(E) Section adjacent to the one shown in (D) was immunostained with anti-NKX 3.1 antibody (brown) and counterstained with methyl green (light blue). Nuclear NKX 3.1 staining was localized to the outgrowing prostatic ducts and the peripheral region of urogenital sinus epithelium. Scale bar, 50 μm.

Figure 4.

GFP expression during the postnatal development of prostate in PSCA-GFP transgenic mice.

Transillumination image of the microdissected lateral prostate from 1 (A), 5 (B), 8 (C), or 14 (D) week old transgenic mice. P, proximal end of prostate, which is connected to urethra. Scale bar, 200 μm.

Figure 5.

GFP expression during regression and regeneration of prostate in PSCA-GFP transgenic mice.

(A-F) Transillumination (TI, left panel) or green fluorescent (GF, right panel) images of the lateral prostate from a 14 week old intact transgenic mouse (A), a 14 week old transgenic mouse that had been castrated for 2 weeks (B), a 14 week old transgenic mouse that had been castrated for 2 weeks then implanted with an androgen pellet for 1 day (C), a 14 week old transgenic mouse that had been castrated for 2 weeks then implanted with an androgen pellet for 7 days (D), a 18 week old transgenic mouse that had been castrated for 2 weeks then implanted with an androgen pellet for 5 weeks (E), or a 14 week old transgenic mouse that had been implanted with an androgen pellet for 7 days (F). Scale bar, 200 μm.

Figure 6.

Differential expression of various markers in GFP positive and negative cells of prostates from the PSCA-GFP transgenic mice.

(A, B) Flow cytometry analysis of dissociated lateral prostates from the 8 week old non-transgenic mice (A) and transgenic mice (B). GFP positive and negative cells that were sorted for RNA preparation are shown in the areas marked with green and blue lines, respectively.

(C) Gene expression analysis of sorted cell populations using RT-PCR. RNA samples were prepared from the sorted cell populations, and were subject to RT-PCR analysis. + (left) and – (right) represent GFP positive and negative populations, respectively. ERβ, estrogen receptor β; BMPR1, bone morphogenetic protein type 1 receptor.

Figure 7.

GFP expression during tumorigenesis of prostate in TRAMP/PSCA-GFP transgenic mice.

(A) A urogenital system from a 23 week old GFP+/TRAMP- transgenic mouse (left) or a GFP+/TRAMP+ littermate (right). SV, seminal vesicle; B, bladder.

(B, C) Transillumination (TI, left panel) or green fluorescent (GF, right panel) images of the lateral (B) or dorsal (C) prostate from a 23 week old GFP-/TRAMP- transgenic mouse (left) or a GFP+/TRAMP+ littermate (right in panel).

(D-G) Immunohistochemistry of the prostates from the transgenic mice. Sections of the lateral prostate from a 23 week old GFP+/TRAMP- (G+/T-) transgenic mouse (D), a GFP+/TRAMP+ (G+/T+) littermate (E), or a GFP-/TRAMP+ (G-/T+) littermate (G) immunostained with an anti-GFP antibody (brown) and counterstained with hematoxylin (dark blue). The staining signal shown in (E) was shown to be specific by staining of the section of the lateral prostate from from a 23 week old GFP+/TRAMP+ (G+/T+) transgenic mouse with control rabbit IgG.

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Figure 1.

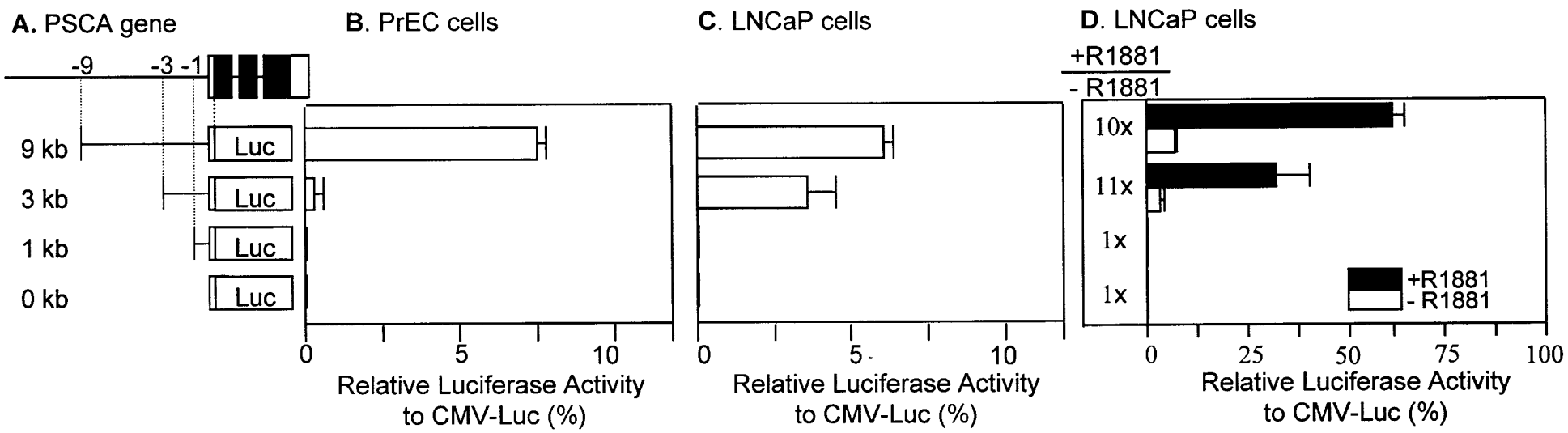

A. PSCA gene
-9
-3
-1
9 kb
3 kb
1 kb
0 kb
Luc
Luc
Luc
Luc
B. PrEC cells
0
5
10
Relative Luciferase Activity
to CMV-Luc (%)
C. LNCaP cells
0
5
10
Relative Luciferase Activity
to CMV-Luc (%)
D. LNCaP cells
+R1881
- R1881
10x
11x
1x
1x
+R1881
- R1881
0
25
50
75
100
Relative Luciferase Activity
to CMV-Luc (%)

Figure 2.

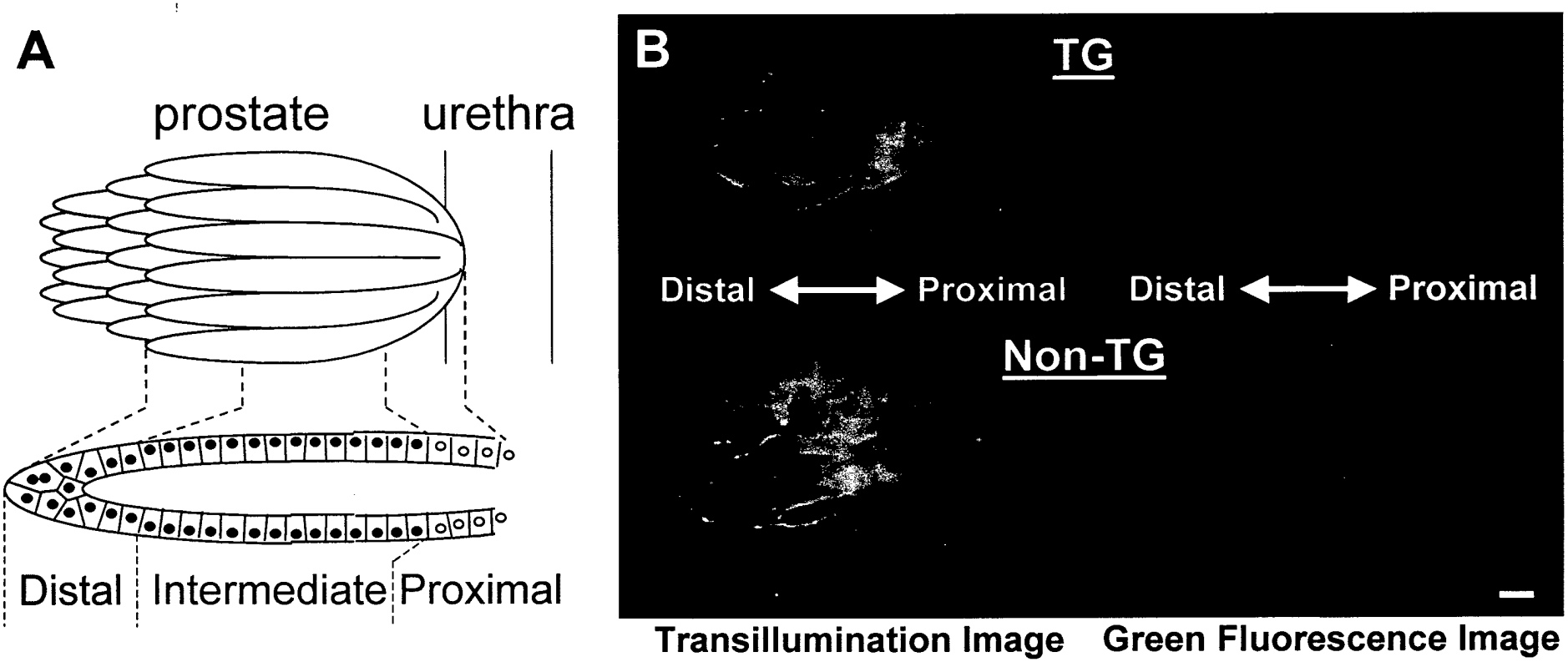
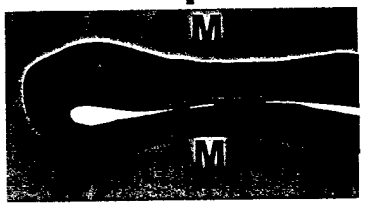
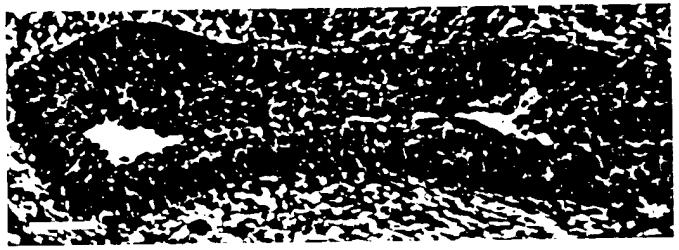

A
prostate
urethra
Distal
Intermediate
Proximal
B
TG
Distal
Proximal
Distal
Proximal
Non-TG
Transillumination Image
Green Fluorescence Image

Figure 3.

A. 16 dpc UGS

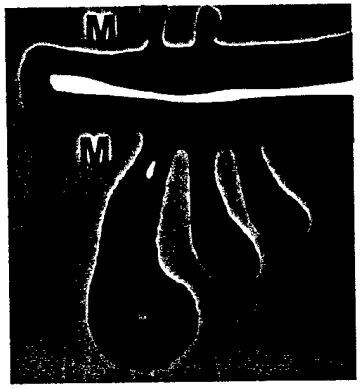


C. α-GFP

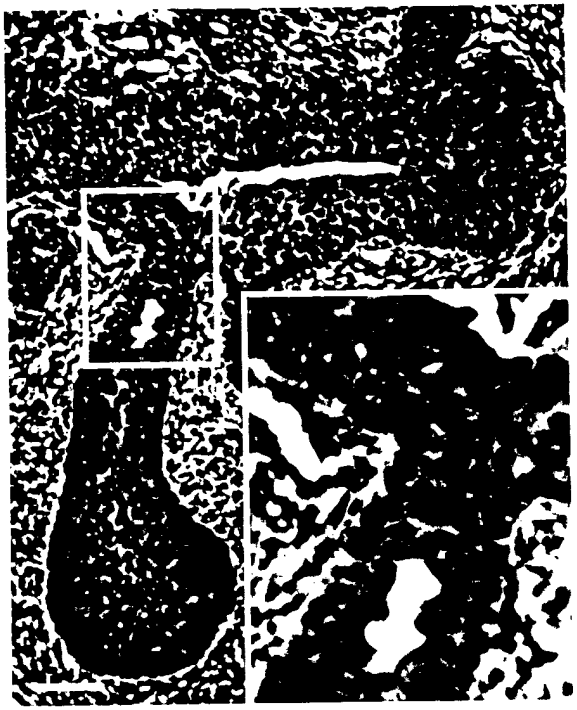


100X

B. 18 dpc UGS

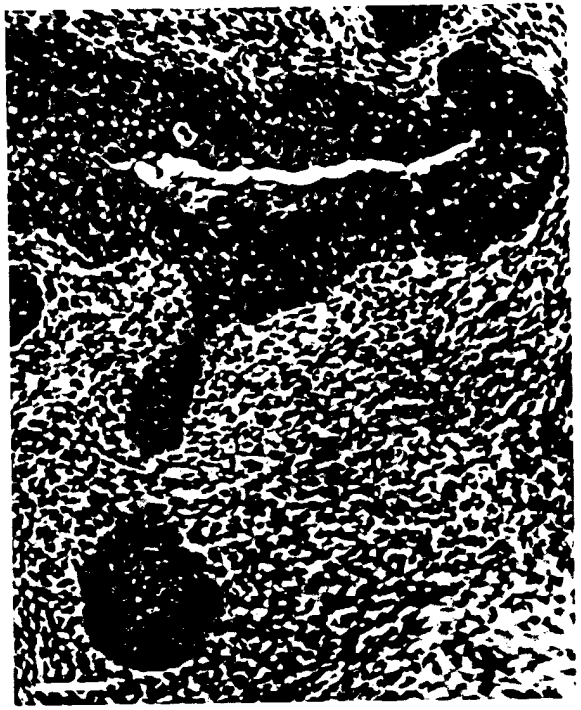


D. α-GFP



100X 250X

E. α-Nkx 3.1



100X

Figure 4.

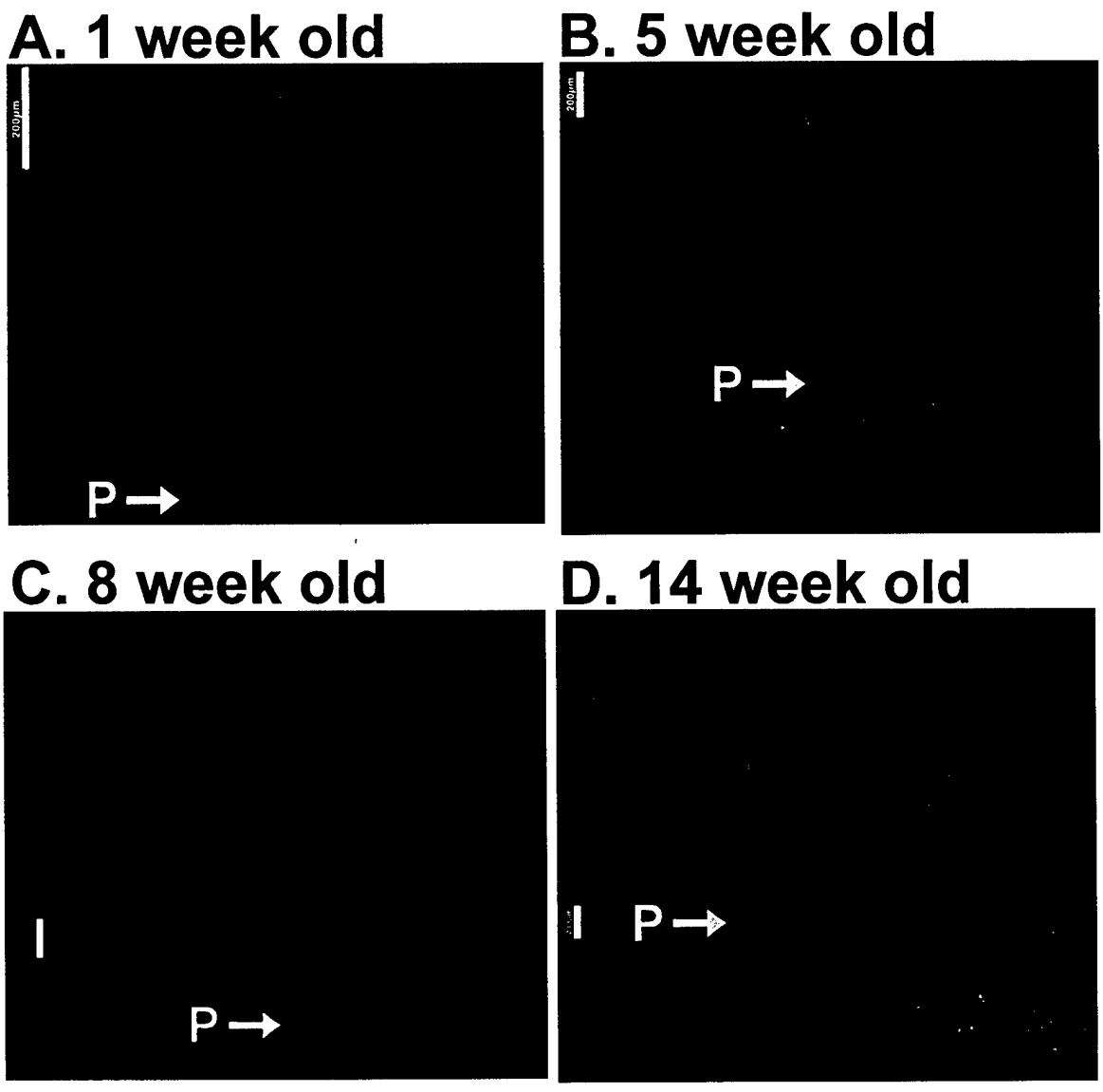

A. 1 week old
200µm
P
B. 5 week old
200µm
P
C. 8 week old
I
P
D. 14 week old
I
P

Figure 5.

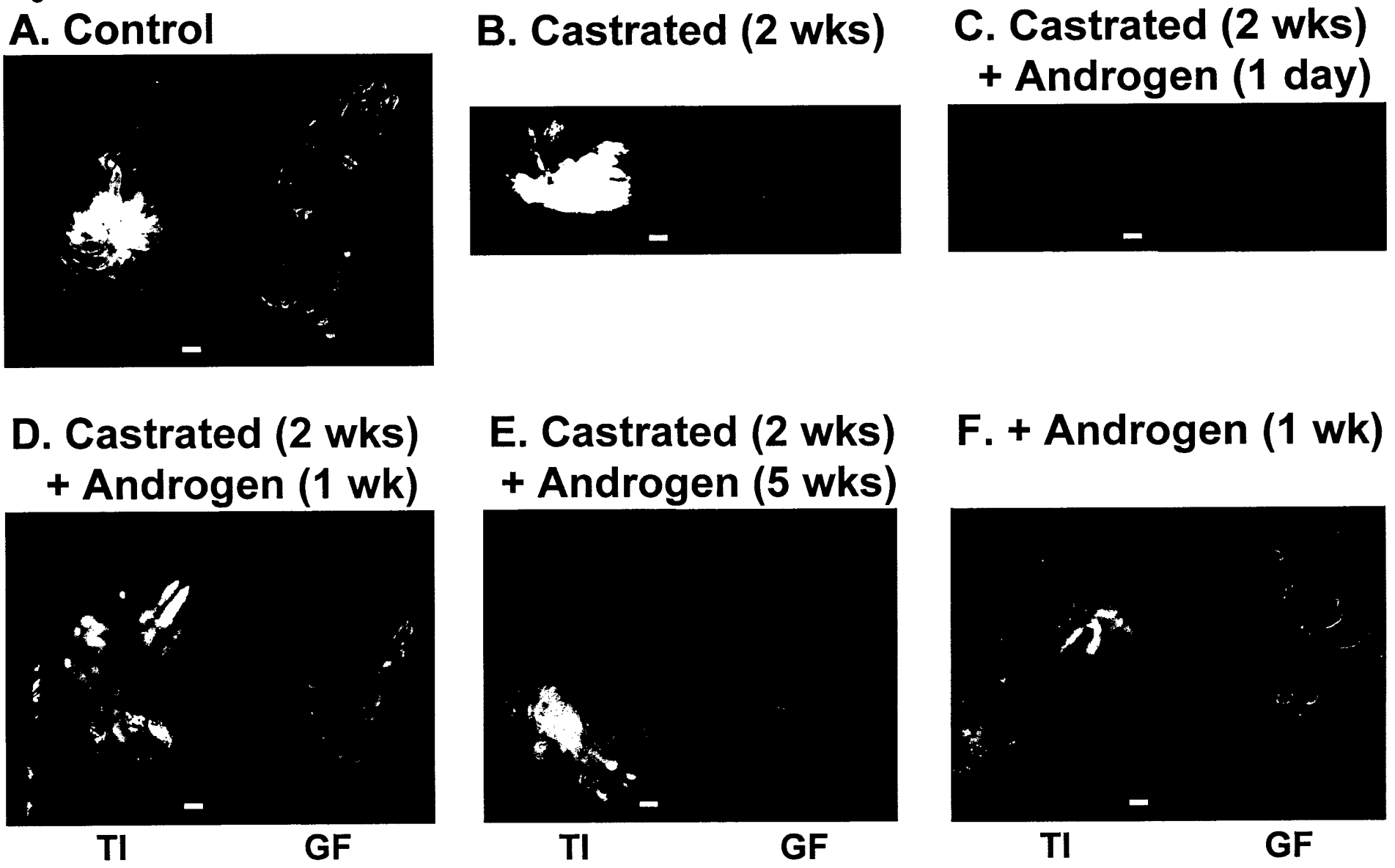

A. Control
B. Castrated (2 wks)
C. Castrated (2 wks) + Androgen (1 day)
D. Castrated (2 wks) + Androgen (1 wk)
E. Castrated (2 wks) + Androgen (5 wks)
F. + Androgen (1 wk)
TI
GF
TI
GF
TI
GF

Figure 6.

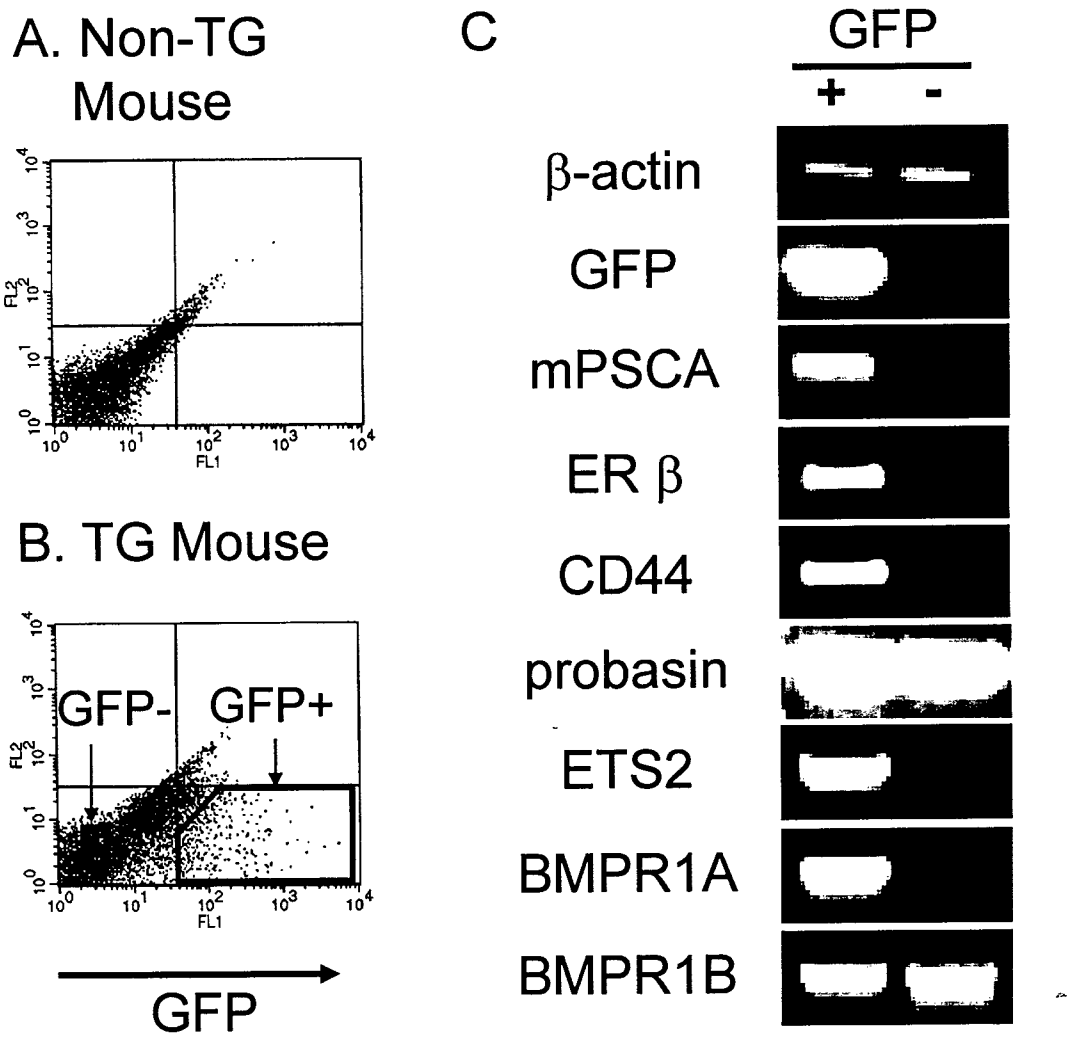
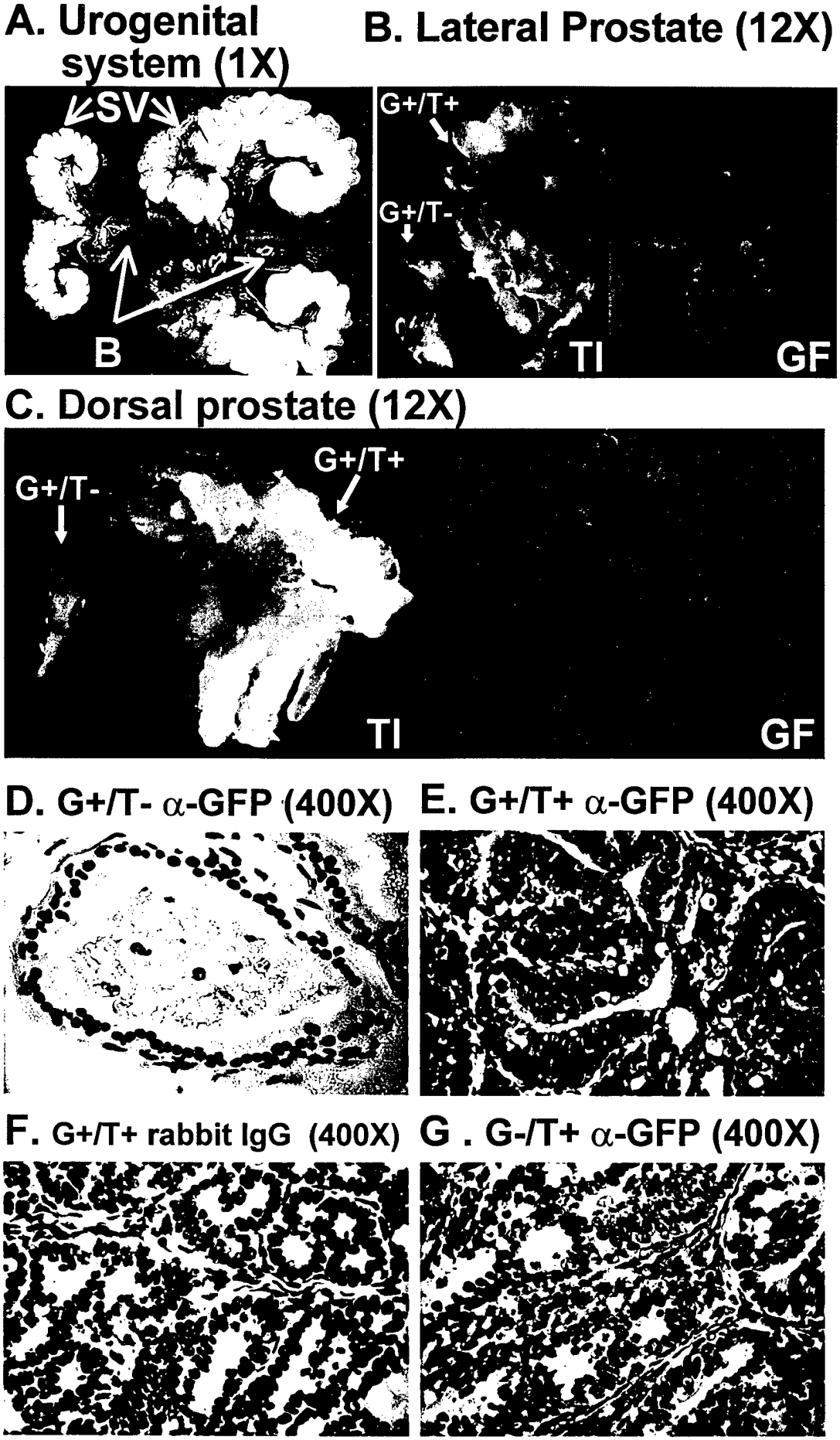

A. Non-TG Mouse
B. TG Mouse
GFP-
GFP+
FL1
FL2
GFP
C
GFP
+
-
β-actin
GFP
mPSCA
ER β
CD44
probasin
ETS2
BMPR1A
BMPR1B

Figure 7.


A. Urogenital system (1X)
SV
B
B. Lateral Prostate (12X)
G+/T+
G+/T-
TI
GF
C. Dorsal prostate (12X)
G+/T+
G+/T-
TI
GF
D. G+/T- α-GFP (400X)
E. G+/T+ α-GFP (400X)
F. G+/T+ rabbit IgG (400X)
G . G-/T+ α-GFP (400X)